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Running head: Obesity and cerebrovascular disease

Abstract

Objective: To systematically investigate causal relationships between obesity and cerebrovascular disease, and the extent to which hypertension and hyperglycemia mediate the effect of obesity on cerebrovascular disease.

Methods: We used summary statistics from genome-wide association studies for body mass index (BMI), waist-to-hip ratio (WHR) and multiple cerebrovascular disease phenotypes. We explored causal associations with two-sample Mendelian randomization (MR) accounting for genetic covariation between BMI and WHR; and assessed what proportion of the association between obesity and cerebrovascular disease was mediated by systolic blood pressure (SBP) and blood glucose levels respectively.

Results: Genetic predisposition to higher BMI did not increase the risk of cerebrovascular disease. In contrast, for each 10% increase in WHR there was a 75% increase (95% confidence interval (CI)=44%-113%) in risk for large artery ischemic stroke, a 57% (CI=29%-91%) increase in risk for small vessel ischemic stroke, a 197% increase (CI=59%-457%) in risk of intracerebral hemorrhage, as well as an increase in white matter hyperintensity volume (β=0.11; CI=0.01-0.21). These WHR associations persisted after adjusting for genetic determinants of BMI. Approximately one tenth of the observed effect of WHR was mediated by SBP for ischemic stroke (12%; CI=4%-20%), but no evidence of mediation was found for average blood glucose.

Interpretation: Abdominal adiposity may trigger causal pathological processes, partially independent from blood pressure and totally independent from glucose levels, that lead to cerebrovascular disease. Potential targets of these pathological processes could represent novel therapeutic opportunities for stroke.

Introduction:

Deaths related to stroke across all ages have seen an increase of 22% in the last decade[1]. Novel therapeutic targets are needed, and their discovery could be propelled by clarification of causal pathways. Observational studies have shown that obese individuals experience roughly double the risk for stroke compared to those of normal weight[2]. Whether this association is causal or a proxy for unmeasured lifestyle factors or covarying health exposures remains unclear. For instance, prior studies have shown that body mass index (BMI) is not associated with stroke risk after adjusting for known vascular risk factors[3]. Observational analyses intending to separate the inherent risk conferred by adiposity from the effect of covarying mediators (such as hypertension) may suffer from confounding and underestimation of true effects[4].

Mendelian randomization (MR) is an instrumental variable analysis approach that has been deployed in genetic epidemiology to determine whether associations of co-occurring traits reflect a causal relationship or simple correlation[5]. Two previous analyses applied MR to show that central adiposity increases risk of ischemic stroke, among other cardiovascular phenotypes[6,7]. Leveraging the most updated genome-wide association study (GWAS) data for body fat distribution and obesity, stroke, and additional cerebrovascular disease traits, we aimed to: (i) determine the degree to which different cerebrovascular disease subtypes are affected by obesity traits; (ii) clarify which obesity trait (waist-to-hip ratio (WHR)

or BMI) best explains any increase risk of stroke; and (iii) assess the role of potential mediators of any identified causal effects.

Methods:

Traits analyzed and genome-wide association studies

Publicly available GWAS summary meta-analysis statistics were obtained for BMI and WHR, and for intracerebral hemorrhage (ICH), white matter hyperintensity (WMH) volume and ischemic stroke. Genetic association results for BMI and WHR were derived from the largest currently available meta-analysis of European ancestry individuals[8]. Briefly, we used summary-level genotype data of both sexes from the UK Biobank and the GIANT consortium, which tested genome-wide associations for WHR and BMI in 694,649 subjects. Phenotypes were adjusted for sex, age and recruitment center and underwent center rank inverse normalization. Ischemic stroke was analyzed *in toto* (all-cause ischemic strokes) and further as subclassified into large-artery atherosclerotic stroke (LAS), cardioembolic stroke (CES), and small-vessel stroke (SVS)[9]. ICH was subclassified into lobar and non-lobar ICH, given the different pathophysiology by location[10]. Genetic data for ischemic stroke (including the etiological subtypes) and for ICH were derived from the MEGASTROKE consortium[9] and the International Stroke Genetic Consortium (ISGC) GWAS for ICH[11], respectively**.** In brief we utilized the results obtained from inverse-variance

meta-analysis restricted to subjects of European ancestry after adjusting for age, sex and principal components reflecting ancestry (40,585 cases; 406,111 controls). To extend the spectrum of cerebrovascular disease phenotypes, we also analyzed WMH volume (in mm^3), a known magnetic resonance imaging (MRI) biomarker of cerebral small vessel disease[12]. We used GWAS summary statistics for MRI volumetric measurements of WMH derived from a UK Biobank study, as previously described[13].

Systolic blood pressure (SBP) and fasting blood glucose (FG) were identified as possible mediators of obesity, based on previous literature demonstrating hypertension and diabetes as being affected by obesity while conferring risk for stroke[6,14,15]. Although abnormal cholesterol and triglyceride levels are also associated with obesity, given inconsistent and sometimes diverging risk associations between lipid levels and stroke risk, we chose to limit mediation analysis to SBP and FG[16]. We prioritized the continuous traits of FG and hemoglobin A1c (HbA1c) over diabetes case/control status, as dichotomous exposure variables can introduce bias into MR analyses related to violation of the monotonicity assumption, as the variants employed as instruments will not predict the exposure phenotype in all individuals[17]. Compared to HbA1c, FG is less affected by erythrocyte lifespan in non-diabetic participants[18] and is informed by a more powered GWAS. FG was therefore selected for the primary analysis while HbA1c was included for confirmation. SBP genetic association data were derived from the UK Biobank. Specifics of the analysis have been detailed elsewhere[19]. Briefly, 317,195 individuals of European Ancestry were included to generate

summary level GWAS data for SBP corrected for the effects of anti-hypertensive treatment. European ancestry-specific genome-wide meta-analysis summary statistics for association with HbA1c and FG were available through the latest effort of the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC, www.magicinvestigators.org)[20,21]. Genotyping and bioinformatic genetic analysis of each of these GWAS followed standardized procedures that are harmonized and comparable across the studies. Detailed genotype and phenotype assessment procedures are available in the studies referred[8-9,11,19-21]. Additional demographic and phenotypic information on the included studies is available in **Table 1**.

Statistical Analysis:

Overall, we used three genetic instruments for our exposures of interest including, BMI, WHR and WHR adjusted for BMI (WHRadj), tested against cerebrovascular disease outcomes. Association estimates for BMI, WHR and outcomes were extracted from the above-mentioned studies, harmonized by risk allele, and used in the MR analyses with the association estimates between the instruments and the described outcomes. The included instruments were single-nucleotide polymorphisms (SNPs) pruned at r^2 <0.01 based on the European 1000-Genomes reference panel, that demonstrated association with BMI and WHR, individually, at genome-wide significance ($p<5\times10^{-8}$).

WHRadj was derived from BMI and WHR. Since BMI is a correlated covariate of WHR, collider bias may be present when using summary statistic data from GWAS of WHR adjusted for BMI[22]. To avoid this problem, we used a

multivariable mendelian randomization model[23], using WHR as a predictor and BMI as a covariate.

Mendelian Randomization Analyses:

Complementary MR approaches were applied. We first used the inverse variance–weighted (IVW) method[24]. Heterogeneity across estimates was assessed using I^2 and Cochran's Q (p<0.05 and I^2>50% were considered statistically significant)[25]. Then, we used the weighted median estimator[26], a method that can provide valid estimates if at least 50% of the information in the analysis comes from SNPs that are valid instrumental variables,[26] and MR-Egger regression[27], generally considered to be conservative in the presence of pleiotropic variants and less likely to generate inflated test statistics leading to false positive associations[28]. As a further sensitivity analysis, we also performed the weighted mode-based estimation method, which provides strongest estimates when the most common causal effect estimate is a consistent estimate of the true causal effect, even if the majority of instruments are invalid[29]. We applied the Benjamini-Hochberg procedure for controlling the false positive rate in multiple comparisons. Finally, when significant MR results between traits were found, we used (i) MR-PRESSO[30], to explore presence of outliers that could bias the results, and (ii) bidirectional MR, to test for any inverse associations using obesity traits as outcomes and cerebrovascular disease phenotypes as exposures[31]. In our bidirectional MR we pruned at r^2 <0.5 using SNPs associated with cerebrovascular disease at genome-wide significance. The higher r^2 threshold for bidirectional MR was required given the reduced number of significant SNPs

associated with cerebrovascular disease. We also adjusted for genetic correlation (based on 1000 Genomes Project CEU population) between instruments by weighting for the inverse LD correlation matrix.

Mediation analysis:

For the mediation analysis, we performed conventional network MR[32]. Specifically, we first estimated the effect of BMI/WHR on each respective mediator (SBP and FG) using the IVW MR approach. Next, we applied regression-based multivariable MR to estimate the effect of SBP and FG respectively on risk of stroke and its subtypes, adjusting for the genetic effect of the instruments on BMI or WHR respectively[27]. The indirect effect of the considered exposure on stroke risk mediated through SBP or FG was estimated by multiplying results from these two MR analyses. We finally divided the mediated effect by the total effect to estimate the proportion mediated, as previously done[19,32]. The above-described method was applied to HbA1c as well.

Analysis was performed using R version 3.4.3 (The R Foundation for Statistical Computing) together with the R packages gtx and MR-PRESSO[30]. The list of genetic variants used appear in **Supplemental Table 1**. This study used publicly available deidentified data from participating studies that had already obtained relevant authorization from local ethics and/or institutional review boards.

Results:

BMI and WHR and cerebrovascular traits

Our first MR approach (IVW-MR) suggested that increased BMI is significantly associated with increased risk of all cerebrovascular traits. This association appeared to be affected by significant heterogeneity as defined by Cochran's Q between SNPs (**Supplemental Table 2**). Similar findings were observed when testing for associations between WHR and cerebrovascular disease. Given concern that pleiotropy may have been contributing to an inflation in association statistics and thus potentially undermining the true causality of the observed relationships, we turned to additional, more conservative MR approaches: Weighted Median MR and Egger regression.

The Weighted Median MR found that each 4.81 kg/m^2 increase in BMI was associated with a modest 0.12 mm^3 increase in WMH volume (β=0.12; 95% Confidence Intervals (CI)= 0.04-0.20; $p=6.00\times10^{-3}$). However, none of the remaining cerebrovascular disease traits were found to be causally affected by BMI using the Weighted Median approach. Egger regression testing for a causal relationship between BMI and WMH was not significant (β=0.04; CI=-0.06-0.14; p=0.742; Egger intercept p=0.068) (**Figure 1A**).

In contrast, findings related to WHR largely retained significance using these conservative MR approaches. Weighted Median MR analyses showed genetically-determined higher WHR to be associated with an increased risk of all-cause ischemic stroke, specifically the LAS and SVS subtypes, with a one standard

deviation increase in WHR of 0.09 (9% increase in WHR absolute, 10% relative to the mean) corresponding to an increase in LAS and SVS by 75% and 57%, respectively. Genetically-determined higher WHR also increased the risk of non-lobar ICH and WMH; the same 9% increase in WHR increased non-lobar ICH risk by 197% and WMH volume by 0.11 mm^3. No associations were found for CES or lobar ICH (**Figure 1B**). Reassuringly, the Egger regression approach was concordant with the Weighted Median MR results for the causal relationship between WHR and all stroke (Odds Ratio (OR)=1.31; CI=1.10-1.56; *p*=0.018; Egger intercept p=0.892), LAS (OR=1.63; CI=1.04-2.56; *p*=0.045; Egger intercept *p*=0.776) and SVS (OR=1.58; CI=1.05-2.39; *p*=0.045; Egger intercept *p*=0.923). For non-lobar ICH and WMH, Egger regression analysis testing the causal effect of WHR returned similar effect size estimates to the Weighted Median MR approach but they were not statistically significant (non-lobar ICH: OR=3.10; CI=0.67-14.28; *p*=0.168; Egger intercept *p*=0.903; WMH: β=0.17; CI= -0.02-0.35; *p*=0.117; Egger intercept *p*=0.663).

Sensitivity analyses

As a further test of the robustness of our findings across MR methodologies, weighted Mode-based MR analysis showed consistent effect sizes and directions to those from the primary Weighted Median MR (**Supplemental Table 2**). MR-PRESSO did not identify any outliers and further supported a lack of bias related to pleiotropy between WHR and all-cause ischemic stroke, LAS and SVS (**Supplemental Table 2**). When applying bidirectional MR, we found no significant associations between the cerebrovascular disease phenotypes and

WHR (**Supplemental Table 3**), supporting our modeling assumption that genetic determinants of stroke risk are unlikely to causally influence WHR.

WHR adjusted for BMI

Multivariable MR demonstrated that WHR causally influenced cerebrovascular risk even after controlling for BMI. An increase in WHR (1 standard deviation, 9% absolute or 10% relative increase from the mean) adjusted for BMI was independently associated with all-cause ischemic stroke (OR=1.25; CI=1.15-1.35; $p=1.12\times10^{-6}$); LAS (OR=1.72; CI=1.38-2.13; $p=1.24\times10^{-6}$), SVS (OR=1.93; CI=1.59-2.35; $p=3.80\times10^{-10}$); non-lobar ICH (OR=2.78; CI=1.34-5.78; $p=6.0\times10^{-3}$) and WMH (β= 0.10; CI=0.08-0.28; $p=3.79\times10^{-4}$), but not with CES or lobar ICH, supporting the results of our univariate MR models for WHR.

Mediation analysis

The effect of WHR was partially mediated by SBP for all-cause ischemic stroke (proportion mediated: 12%; CI=4%-20%; $p=5.0\times10^{-3}$); LAS (proportion mediated: 13%; CI=2%-25%; $p=0.03$); and SVS (proportion mediated: 11%; CI=1%-21%; $p=0.04$). We did not find a significant mediation effect for non-lobar ICH (proportion mediated: 9%; CI=-5%-23%; $p=0.235$) and WMH (proportion mediated: 12%; CI=-7%-31%; $p=0.210$) (**Figure 2**). Mediation analyses for the effect of FG on WHR-mediated risk did not show significant effects for all-cause ischemic stroke (proportion mediated: 0%; CI=-38%-16%; $p=0.430$); LAS (proportion mediated: 3%; CI=-22%-29%; $p=0.810$); SVS (proportion mediated: 18%; CI=-12%-48%; $p=0.251$); non-lobar ICH (proportion mediated: 1%; CI=-

20%-22%; p=0.909) or WMH (proportion mediated: 0%; CI=0%-100%; p=0.840). Similar results were found when testing HbA1c instead of FG.

Although the weighted median MR approach identified a potential causal effect for BMI on WMH volumes alone, and at a smaller effect size than what has been observed in association with clinical outcomes, in the interest of completeness we also explored mediating factors for any effect of genetically-determined BMI (as an exposure) on cerebrovascular disease. SBP significantly mediated a considerable part of the putative association between BMI and all-cause ischemic stroke, LAS and SVS (**Figure 2**). FG and HbA1c did not mediate BMI risk on any tested cerebrovascular phenotypes (**Supplemental Table 4**).

Discussion:

Leveraging GWAS summary data, our study confirms a causal relationship between obesity and cerebrovascular disease, relates it primarily to abdominal adiposity and specific cerebrovascular phenotypes, and clarifies a possible role for mediation through hypertension but not hyperglycemia.

Several previous observational studies have investigated the association between obesity and risk of ischemic strokes[34–36]. Both previous MR analyses[6,7] suggested that obesity, but not BMI, could be causal in this relationship, showing an overall 30% increased risk for ischemic stroke for each standard deviation increase in abdominal adiposity[6]. However, the studies failed to confirm the association when more catered for unbalanced horizontal pleiotropy. Lastly, both previous studies

did not resolve which ischemic stroke subtypes are specifically affected by abdominal adiposity. Our analyses, leveraging greater statistical power enabled by the latest consortia efforts, are able to confirm these results with MR approaches more robust to the presence of pleiotropic instruments[28], suggesting that higher abdominal adiposity intrinsically increases risk of ischemic stroke. Our study also confirms the lack of a robust causal effect for BMI across ischemic stroke and its subtypes. As has been previously suggested, BMI ignores important health-determinants such as muscle mass and distribution of adiposity, and may therefore be a relatively poor biological tool for examination of causal pathways in disease[37,38]. Our results suggest that WHR specifically increases stroke risk, and this effect persists even after adjustment for BMI. Given that studies with direct assessments of body adiposity showed that WHR adjusted for BMI is a surrogate measure of abdominal adiposity[39] our results specifically implicate abdominal distribution of fat in a causal process leading to cerebrovascular disease.

Two prior genetic studies have investigated the relationship between obesity and ICH and did not identify any significant associations[6,7]. To our knowledge, no study has investigated distinct effects between lobar and non-lobar ICH. In contrast to ischemic stroke, epidemiologic studies have reported conflicting results on the association between obesity and ICH risk, with one study reporting an increased prevalence of obesity in patients hospitalized for ICH[40], and another showing that ICH cases have lower BMI[41]. In another multicenter study, BMI increased the risk of non-lobar ICH but only through an indirect effect of hypertension[42]. Our results support WHR but not BMI as a risk factor for non-

lobar ICH alone, with no contribution to risk of lobar ICH. Although we cannot fully exclude horizontal pleiotropy in this situation, all the employed MR approaches, which make distinct assumptions on the presence of any pleiotropic variants, returned consistent results. Finally, studying the effect of adiposity on white matter lesions, we found that even if genetic susceptibility for higher BMI and WHR are statistically associated with more severe WMH, the overall effect is negligible and is unlikely to be clinically impactful. Given the common small vessel pathology shared between non-lobar ICH, SVS, and WMH[12], it may be reasonable to hypothesize that abdominal adiposity may prompt pathological mechanisms that also lead to WMH. Deployment of higher power GWAS of WMH in healthy subjects or use of WMH GWAS data from subjects affected by cerebrovascular disease may yield different results.

Research has suggested several pathological cascades arising from obesity that lead to thrombosis and cardiovascular disease. The most intuitive are represented by increased blood pressure and diabetes, two recognized causal factors for arteriosclerosis of large and small blood vessels[12]. Compared to mediation analysis based on observational data, MR is less susceptible to measurement error[19] and therefore offers favorable opportunities for understanding any mediation by hypertension and hyperglycemia in obesity. Our mediation results suggest that causal mechanisms arising from abdominal fat distribution contribute to cerebrovascular disease largely independent of these factors. Along with metabolic derangements, obesity is associated with significant and protracted increase in inflammatory markers, and as such it has been described as a low-

grade chronic inflammatory state[43]. Low grade inflammation and systemic oxidative stress have proven to be damaging for the endothelium, shifting it towards a prothrombotic state. Platelet reactivity, enhanced coagulation and impaired fibrinolysis are other recognized mechanisms[44]. Our results demonstrating increased risk for SVS and non-lobar ICH on one hand, and for LAS on the other suggest that both cerebral microvascular disruption and accelerated atherosclerosis leading to stroke may be triggered by obesity.

Our MR benefits from multiple and orthogonal approaches. Although results from different approach were comparable in direction and effect size, association results from IVW-MR appeared to be inflated and affected by pleiotropy. Therefore, we focused on the more conservative and selective approach of Weighted Median and Egger regression, more robust to the inclusion of pleiotropic variants. Mode-based results were also consistent but produced less precise estimates and wider confidence intervals, perhaps reflecting a poorer model-fit for modal assumptions.

Our study has several limitations. Although we have used multiple MR approaches to guard against confounding due to pleiotropy, we cannot fully exclude residual bias, which is an established limitation of the MR approach[45]. Similarly, we attempted to limit the misleading inferences introduced by trait heterogeneity by applying multivariable MR. Well-powered studies with individual level data on the included small vessel phenotypes in addition to measured BMI and WHR values will ultimately be required in order to confirm the causal effects of complex phenotypes like adiposity. Further, our study is

limited to individuals of European ancestry and as such our results cannot necessarily be generalized to other ancestral populations. This is particularly relevant given the known disparities in obesity and stroke risk in traditionally underserved populations such as blacks and Hispanics[46]. Future studies building on our approach in diverse populations are needed to extend our findings. We did not include genetic predisposition to lipid blood levels as a mediator of the relationship between obesity and cerebrovascular disease, given that lipid effects on cerebrovascular outcomes are unsettled and potentially opposing in effects across ischemic and hemorrhagic stroke. Although triglyceride and cholesterol levels are related to central obesity, associations between cholesterol levels and increased risk of stroke lack the consistency of studies on hypertension and diabetes,[47] and associations in ICH are even more unclear[48]. As such, the results of a lipid mediation analysis would be difficult to interpret. Lastly, individual level data were not available; this precluded us from extending our analysis to sex-specific effects of obesity. However, previous observations showed that sexual dimorphism affects SNPs associated with WHR less than the ones associated with WHRadj[8].

Conclusions:

Our study identifies abdominal adiposity as a causal risk factor for cerebrovascular disease, demonstrates differential effects across stroke subtypes, and suggests a substantial proportion of this effect extends beyond hypertension and diabetes. These results support the pursuit of pathological targets induced by

central obesity as potential therapeutic candidates for stroke[49]. The importance of this topic is amplified by the ongoing obesity epidemic around the world[50].

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Author Contributions:

Conception and design of the study: CDA, DG, JM, SM.

Acquisition and analysis of data: DG, BEM, JM, RM, SM, CLS.

Drafting a significant portion of the manuscript or figures: CDA, DG, JCF, BEM, SM, MD, JR.

Potential Conflicts of Interest:

None.

Figure Captions

Figure 1: Mendelian randomization associations of genetically determined BMI and WHR with cerebrovascular disease. Results derived from weighted median Mendelian Randomization analysis (Odds Ratio and 95% Confidence intervals for each 6% increment in BMI and 9% increment in WHR, corresponding to one standard deviation in each) are shown.

Legend: BMI: body mass index; WHR: waist hip ratio; WMH: white matter hyperintensity; ICH: intracerebral hemorrhage; LAS: large artery stroke; SVS: small vessel stroke; CES: cardio embolic stroke.

Figure 2: Mediation analysis: estimates for the SBP mediating the effect of WHR and BMI on cerebrovascular disease outcomes. For any cerebrovascular disease, we report the average proportion mediated (dot), the 95% confidence intervals of the percentage mediated (grey bar) and p value.

Legend: BMI: body mass index; WHR: waist hip ratio; WMH: white matter hyperintensity; ICH: intracerebral hemorrhage; LAS: large artery stroke; SVS: small vessel stroke; CES: cardio embolic stroke.

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Table 1: Characteristics of subjects included in the GWAS studies utilized for the analyses**.**

	TRAIT	CONSORTIUM	Subjects	Women (%)	mean age (SD)	Trait mean value (SD)
Obesity traits	BMI	UKBiobank+GIANT	806,834	54	NA	27.41 (4.81)
	WHR		697,734	55	NA	0.87 (0.09)
			cases			
Cerebrovascular disease phenotypes	IS	MEGASTROKE	10,307	41.7	67.4 (12.3)	
	LAS		3,808	48.9	65.9 (10.4)	
	CES		3,697	46.4	68.1 (9.4)	
	SVS		2,206	45.5	65.6 (12.4)	
	ICH	ISGC	1,545	45.1	67.0 (10)	
	WMH	UK-Biobank	10,597	52.7	54.9 (7.5)	4,607 (6,021) mm^3
Mediation analysis traits	FG	MAGIC	133,010	46.3	50.1 (18.4)	5.33 (0.44) mmol/l
	HbA1c	MAGIC	123,665	51.37	53.8 (10.3)	5.38 (0.22) %
	SBP	UKBiobank	317,195	53.71	56.9 (8.0)	136.39 (18.68)

Legend: BMI: Body Mass Index; WHR: Waist Hip Ratio; IS: Ischemic Stroke; LAS: Large Artery Stroke; CES: Cardioembolic Stroke; SVS: Small-Vessel Stroke; ICH: Intracerebral Hemorrhage, WMH: White Matter Hyperintensity; FG: fasting glucose; HbA1c: hemoglobin A1c